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A Theoretical Study of Discriminating Parameters in Metabolic Resistance to Insecticides

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Abstract: In the case where resistance to an insecticide is associated with increased metabolism of the insecticide, it should not be concluded that the resistance is due only to the increased metabolism (i.e. metabolic hypothesis). Here, we study theoretically the pharmacokinetic consequences of a resistance mechanism due to increased metabolism. We consider two cases: treatment with the initial dose D_0 applied to the susceptible strain and the treatment with the initial dose αD_0, with $\alpha > 1$, applied to the resistant strain. We show the conditions for which the metabolism hypothesis is conceivable. The time τ, from which the mortality of the susceptible strain is significantly higher than that of the resistant strain, is an important parameter in determining the validity of the metabolic hypothesis. The more τ increases, the more the conditions are favourable to this hypothesis. Our work suggests an approach to test the metabolic hypothesis from experimental results. © 1998 SCI

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Key words: insecticide; mathematical modelling; resistance; metabolism; pharmacokinetics

1 INTRODUCTION

Insects cause serious problems in many areas such as crop production, and animal and human health. Insecticides are used but their repeated use leads to the emergence of resistance that reduces their efficacy.[1] The efficacy of an insecticide is due to it binding at target molecules in the insect. However, the insect may modify the molecule, leading to decreased toxicity and increased polarity, which results in better elimination of the insecticide. Generally, resistance results from one of a number of phenomena, including target molecule modification,[2] increased metabolism,[3] and decreased penetration,[4] or from a combination of several of these.

When increased metabolism is associated with insecticide resistance, it is difficult to know whether such increase is the only cause of resistance or whether the resistance has multiple causes. Generally, the resistance ratio (ratio of the LD_{50} for the resistant strain to the LD_{50} for the susceptible strain) and the factor of increase of metabolism (ratio of the velocity constant of metabolism of insecticide in the resistant strain to the velocity constant of metabolism of insecticide in the susceptible strain) are studied. When the values of both ratios are similar, it is often concluded that resistance is due solely to increased metabolism. This conclusion can be premature unless the coherence between the pharmacokinetic consequences which follow from the resistance metabolism and the mortality kinetics has been accurately verified or an extensive genetic analysis has been performed. It is therefore necessary to study these different kinetics.

The aim of this theoretical work is to study the mortality kinetics and the pharmacokinetics to test the conditions under which increased metabolism alone is

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sufficient to explain the resistance. The hypothesis of a resistance mechanism due only to increased metabolism is called the metabolic hypothesis. In previous work, we have developed a method to test the mechanism of synergy between two toxic agents.[5–7] Following the same principle, we here study the conditions under which the metabolism hypothesis is valid and we suggest a possible experimental approach. The validity condition is based on the coherence between the mortality kinetics and the pharmacokinetics simulations. The simulations are obtained from compartmental models in continuous time.

2 METHODS

2.1 Mortality kinetics

The validity of the metabolic hypothesis is studied by comparing the simulated pharmacokinetics and the standard theoretical mortality kinetics (Fig. 1). The four treatments are: control treatment for a strain of susceptible insects (C_S), control treatment for a strain of resistant insects (C_R), insecticide at the dose D_0 for a strain of susceptible insects (D_S), and insecticide at the dose αD_0 (with $\alpha > 1$) for a strain of resistant insects (αD_R).

The value α represents the ratio of the dose applied to the susceptible strain to the dose applied to the resistant strain. The value of α is chosen from the mortality kinetics. The value of α must be the highest for which the mortality of susceptible insects diverges rapidly from that of resistant insects and always stay significantly higher than that of resistant insects.

The mortalities induced by the D_S and αD_R treatments are significantly higher than those induced by the controls C_S and C_R treatments. The mortalities induced by the D_S and αD_R treatments first increase; they then reach a plateau where the instantaneous mortalities are nearly the same for all treatments. After time τ, the mortality induced by the D_S treatment is always higher than that induced by the αD_R treatment.

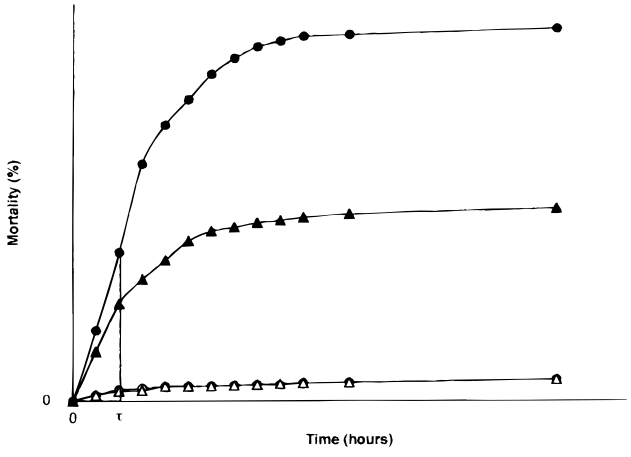

Fig. 1. Time course of mortality produced by different doses of insecticide in susceptible and resistant insect strains. (○) controls for susceptible strain of insect (C_S), (△) controls for resistant strain of insect (C_R), (●) insecticide at a dose D_0 for susceptible insect strain (D_S), (▲) insecticide at a dose αD_0 (with $\alpha > 1$) for resistant insect strain (αD_R).

2.2 Model

The determinist model with two compartments describes the pharmacokinetics of the insecticide. The two compartments represent the external insecticide and the internal insecticide (Fig. 2). The external insecticide (X_1) penetrates the insect body. The internal insecticide (X_2) is metabolised. We choose to group together the excretion of the unmetabolised insecticide and the metabolism phenomenon. The model is thus simplified. The penetration flow is determined by the mass action law

$$X_1 \underset{rk_{+1}}{\overset{k_{+1}}{\rightleftharpoons}} X_2$$

where k_{+1} and rk_{+1} are the velocity constants. In other words, r is the ratio of the velocity constant of the insecticide flow from X_2 to X_1 to the velocity constant of the flow from X_1 to X_2. The system is described by the following equations:

$$\frac{dX_1}{dt} = -k_{+1}X_1 + rk_{+1}X_2$$

$$\frac{dX_2}{dt} = +k_{+1}X_1 - (k_2 + rk_{+1})X_2$$

Variables X_1 and X_2 are expressed in moles, and k_{+1} and k_2 in time^{-1} with $k_{+1} > 0$, $k_2 \geqq 0$ and $r \geqq 0$. Constant k_{+1} denotes the ratio of the fraction of the total quantity of X_1 moving towards compartment X_2 per unit of time to X_1. Similarly, rk_{+1} represents the ratio of the fraction of the quantity of X_2 that moves towards compartment X_1 per unit of time to X_2. The constant k_2 is the ratio of the fraction of the quantity of X_2 per unit of time that is metabolised to X_2.

We consider that the metabolites of the insecticide have no toxicity, as it is the case with pyrethroids.[8] The model is not adapted to insecticides with metabolites

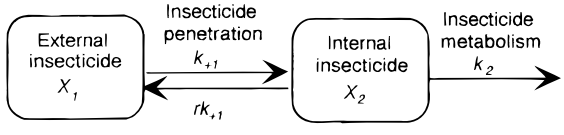

Fig. 2. Schematic representation of the compartmental model for pharmacokinetic study of insecticide. The external insecticide (X_1) penetrates the insect body. Internal insecticide (X_2) is metabolized. Constant k_{+1} denotes the ratio of the fraction of quantity of X_1 moving towards compartment X_2 per unit of time on X_1. Similarly, rk_{+1} represents the ratio of the fraction of quantity of X_2 that moves towards compartment X_1 per unit of time on X_2, and k_2 is the ratio of the fraction of quantity of X_2 that is metabolised per unit of time on X_2.

more toxic than the initial product (e.g. malathion),[9,10] because it would be necessary to include the metabolite compartment in the model. We focus on the compartment X_2 which represents the internal insecticide, as we assume that the mortality depends on the concentration of internal insecticide.

This model is equivalent to the model developed by Ford and Greenwood[11,12] and which was used to describe the pharmacokinetics of various insecticides in different insects.[12–16] We suppose that the resistant strain has only an increased metabolism (a-fold higher).

$$\frac{dX_1}{dt} = -k_{+1}X_1 + rk_{+1}X_2$$

$$\frac{dX_2}{dt} = +k_{+1}X_1 - (ak_2 + rk_{+1})X_2$$

The coefficient a is higher than 1. The coefficient a represents the factor of metabolism increase.

By integration, this gives, in the case of the susceptible strain, the following result:

$$X_1 = -\frac{D_0(\lambda_2 + k_{+1})e^{\lambda_1 t}}{\lambda_1 - \lambda_2} + \frac{D_0(\lambda_1 + k_{+1})e^{\lambda_2 t}}{\lambda_1 - \lambda_2}$$

$$X_2 = \frac{D_0 k_{+1} e^{\lambda_1 t}}{\lambda_1 - \lambda_2} - \frac{D_0 k_{+1} e^{\lambda_2 t}}{\lambda_1 - \lambda_2}$$

Constant D_0 is the initial dose of insecticide applied to the insect. Eigenvalues $\lambda_{1,2}$ are:

$$\lambda_{1,2} = \frac{-(k_{+1} + k_2 + rk_{+1}) \pm \sqrt{(k_{+1} + k_2 + rk_{+1})^2 - 4k_{+1}k_2}}{2}$$

In the case of the resistant strain, the solutions X_1 and X_2 are:

$$X_1 = -\frac{\alpha D_0(\lambda'_2 + k_{+1})e^{\lambda'_1 t}}{\lambda'_1 - \lambda'_2} + \frac{\alpha D_0(\lambda'_1 + k_{+1})e^{\lambda'_1 t}}{\lambda'_1 - \lambda'_2}$$

$$X_2 = \frac{\alpha D_0 k_{+1} e^{\lambda'_1 t}}{\lambda'_1 - \lambda'_2} - \frac{\alpha D_0 k_{+1} e^{\lambda'_2 t}}{\lambda'_1 - \lambda'_2}$$

With

$$\lambda'_{1,2} = \frac{-(k_{+1} + ak_2 + rk_{+1}) \pm \sqrt{(k_{+1} + ak_2 + rk_{+1})^2 - 4ak_{+1}k_2}}{2}$$

and αD_0 the initial dose.

2.3 Test

In order to determine conditions for which the metabolic hypothesis is valid, we study the pharmacokinetics of the insecticide for conditions corresponding to D_S and αD_R treatments. To illustrate both treatments using simulation (Fig. 3), we selected the constants corresponding to a realistic situation (penetration half-life of 1 h with $r = 0$, metabolism half-life of 2 h, initial dose of 20 pmol, $\alpha = 20$ and $a = 20$). In the case of D_S treatment, the initial dose is D_0 and the velocity constant of metabolism is k_2 because the metabolism of the susceptible strain is normal. In the case of αD_R treatment, the initial dose is αD_0 and the velocity constant of metabolism is ak_2 because the metabolism of the resistant strain is increased. Hence, at the start of intoxication, the internal insecticide in the case of αD_R increases faster than that in the case of D_S treatment because the initial dose is α-fold higher. Conversely, the internal insecticide in the case of αD_R treatment decreases sooner and faster than that in the case of D_S treatment because the metabolism is a-fold higher. At the time t_1, the internal insecticide in the case of αD_R treatment is equal to that in the case of D_S treatment. According to the model in which increased metabolism is the only protection, before the time t_1, the internal insecticide in the case of D_S treatment is always lower than that in the case of αD_R treatment. Consequently, since we assume that the motality depends on the internal insecticide, before the time t_1, the mortality in the case of D_S treatment should not be higher than that in the case of αD_R treatment. If, before the time t_1, the mortality in the case of D_S treatment is higher than that in the case of αD_R treatment, then the metabolic hypothesis is rejected. In the opposite case (i.e. mortality in the case of D_S treatment is higher than that in the case of αD_R treatment after the time t_1), the metabolic hypothesis is conceivable. However, we postulated that after the time τ, the mortality of D_S treatment is always

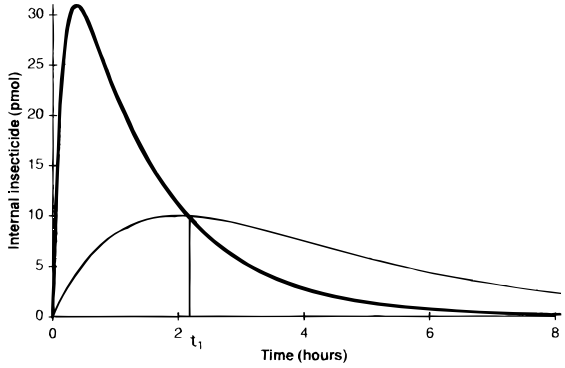

Fig. 3. Simulations of time course of internal insecticide with different initial doses in susceptible and resistant insect strains. In this simulation, the value of penetration half-life is fixed at 1 h so that $k_{+1} = \ln 2\ h^{-1}$. The value of metabolism half-life is fixed at 2 h so that $k_2 = (\ln 2)/2\ h^{-1}$. The values of $r = 0$ (rk_{+1} defined as in Fig. 2), the initial dose $D_0 = 20$ pmol. (——) insecticide at at dose D_0 for susceptible insect strain (D_S), (——) insecticide at a dose αD_0 (with $a > 1$) for resistant insect strain (αD_R). In this simulation, α is fixed at 20 and a (value of coefficient of increase of metabolism of insecticide) is fixed at 20.

significantly higher than that of αD_R treatment. Then, t_1 must be smaller than τ. If $t_1 < \tau$, the metabolic hypothesis is conceivable. If $t_1 > \tau$ the metabolic hypothesis is excluded because there is no coherence between the simulation of the insecticide pharmacokinetics and the mortality kinetics.

The case $t_1 = \tau$ represents the limit between the conditions for which the metabolic hypothesis is excluded and those for which the metabolism hypothesis is conceivable. The conditions for which $t_1 = \tau$ necessarily depend on the value of τ obtained from the mortality kinetics. They also depend on k_{+1}, k_2, and r, which themselves depend on the insecticide, on the insect, on α obtained from the mortality kinetics and on a that represents the increase of metabolism.

3 RESULTS

To determine the conditions for which the metabolic hypothesis is conceivable or not, we study the conditions for which $t_1 = \tau$. First, the study is done with fixed values of τ and α, and then we vary the values of τ and α.

3.1 Representation of the equality $t_1 = \tau$

In order to represent the conditions for which $t_1 = \tau$, we choose to fix the values of a and r, and to vary the values of k_{+1} and k_2. It is possible to represent $t_1 = \tau$ on a plan. To define this plan, we choose $(\ln 2)/k_{+1}$ and $(\ln 2)/k_2$ as axes, instead of k_{+1} and k_2. When $r = 0$, $(\ln 2)/k_{+1}$ represents the penetration half-life and $(\ln 2)/k_2$ the metabolism half-life. We choose these two axes because 'half-lives' are often used in pharmacokinetics and are more evocative than velocity constants.

The $t_1 = \tau$ curve is represented in Fig. 4, with $\tau = 2$ h, $\alpha = 15$, $a = 20$ and $r = 0$. For the points located above the $t_1 = \tau$ curve, the metabolic hypothesis is excluded because $t_1 > \tau$. On the other hand, for the points located under the $t_1 = \tau$ curve, the metabolic hypothesis is conceivable because $t_1 < \tau$. It means that for an insecticide with known k_{+1} and k_2 values, if the point with coordinates corresponding to $(\ln 2)/k_{+1}$ and $(\ln 2)/k_2$ is located under the $t_1 = \tau$ curve, then the metabolic hypothesis is conceivable. If the point is located above the $t_1 = \tau$ curve, then the metabolic hypothesis is excluded. In other words, for given values of α, of a and of τ, if metabolism in the susceptible strain is too slow, the hypothesis is rejected because the increased metabolism in the resistant strain is not sufficient to explain an early mortality at the time τ.

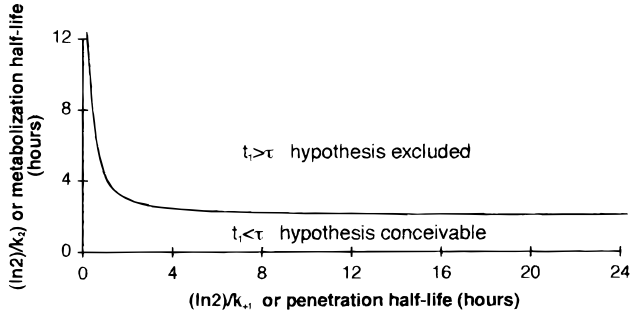

Fig. 4. Limit for which $t_1 = \tau$ as a function of k_{+1} and k_2. We assume values of $\alpha = 15$ (value of the coefficient of increase of the initial dose between the treatment for susceptible strain (D_S) and that for resistant strain (αD_R)) and $\tau = 2$ h (from the time τ, the mortality induced by the D_S treatment is significantly higher than that induced by the αD_R treatment). We set up $a = 20$ (value of coefficient of increase of metabolism), $r = 0$ (rk_{+1} defined as in Fig. 2). The tested hypothesis is excluded in the zone for which $t_1 > \tau$.

3.2 Influence of a on $t_1 = \tau$

The coefficient a represents the factor of increase of metabolism of the insecticide in the resistant strain. We consider different values of a (10, 15, 20 and 30) in the same conditions as before (i.e. $\tau = 2$ h, $\alpha = 15$ and $r = 0$). The results are represented in Fig. 5. It should be noted that the more a increases, the larger the zone for which the metabolism hypothesis is conceivable. If $a > \alpha$, with $(\ln 2)/k_{+1}$ tending to infinity, the $t_1 = \tau$ curve tends to a horizontal asymptote. If $a = \alpha$, with $(\ln 2)/k_{+1}$ tending to infinity, the $t_1 = \tau$ curve tends to the $(\ln 2)/k_{+1}$ axis. If $a < \alpha$, there is a maximal value of $(\ln 2)/k_{+1}$ beyond which t_1 is always higher than τ and the metabolic hypothesis is always excluded.

3.3 Influence of r on $t_1 = \tau$

The coefficient r, as we saw before, is the ratio of the velocity constant of the insecticide flow from X_2 to X_1

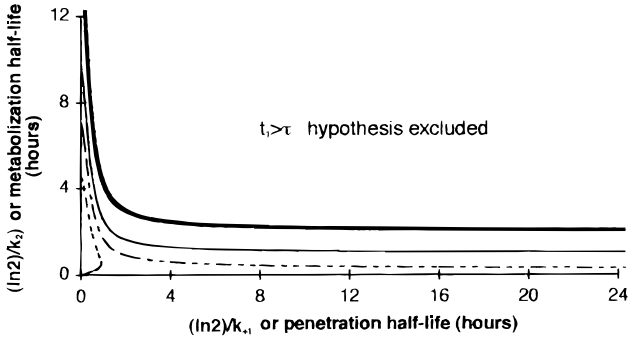

Fig. 5. Limits for which $t_1 = \tau$ as a function of k_{+1} and k_2 with different a values. We assume values of $\alpha = 15$ (value of the coefficient of increase of the initial dose between the treatment for susceptible strain (D_S) and that for resistant strain (αD_R)) and $\tau = 2$ h (from the time τ, the mortality induced by the D_S treatment is significantly higher than that induced by the αD_R treatment). We set up $r = 0$ (rk_{+1} defined as in Fig. 2). The values of the coefficient of increase of metabolism, a are (- - - -) 10, (— - -) 15, (——) 20 and (▬▬) 30. The tested hypothesis is rejected in the zone for which $t_1 > \tau$.

to the velocity constant of the flow from X_1 to X_2. We consider different values of r (0, 0.5, 1 and 1.5) with $\tau = 2$ h, $\alpha = 15$ and $a = 20$. The results are represented in Fig. 6. It should be noted that the more r increases, the larger the zone for which the metabolic hypothesis is excluded. In all cases, the more $(\ln 2)/k_{+1}$ increases (i.e. the more the half-life of penetration increases), the lower the value for which $t_1 = \tau$, beyond which the metabolic hypothesis is excluded.

We consider now that the mortality kinetics of susceptible and resistant strains are different. That is equivalent to varying the values of τ and α.

3.4 Influence of α with $\alpha = a$ on $t_1 = \tau$

The parameter α represents the ratio of the initial dose used for the resistant strain to the initial dose used for the susceptible strain. The parameter α must be the highest for which the mortality of susceptible insects rapidly diverges from that of resistant insects and always stays significantly higher than that of resistant insects. In the case $a = \alpha$, there is the same value for the factor of increase of metabolism (a) and for the ratio between the initial doses (α). We consider different values of α with $a = \alpha$ (15, 20 and 30) with $\tau = 2$ h and $r = 0$ (Fig. 7). The more α increases (with $a = \alpha$), the larger the zone for which the metabolic hypothesis is conceivable. As before, the more $(\ln 2)/k_{+1}$ increases, the lower the value for which $t_1 = \tau$, beyond which the metabolic hypothesis is excluded, and also the closer the $t_1 = \tau$ curves which are obtained for different values of $a = \alpha$.

The study of the different $t_1 = \tau$ curves for different values of α, and with a fixed value of a, would have given results similar to those in Fig. 5. However, the situation is the reverse of that with a in that the more α increases, the larger the zone for which the metabolic hypothesis is excluded.

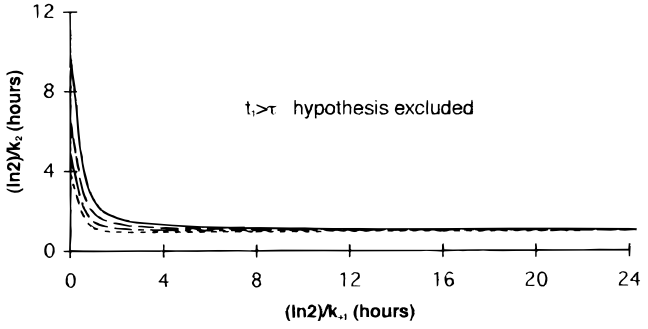

Fig. 6. Limits for which $t_1 = \tau$ as a function of k_{+1} and k_2 with different r values. We assume values of $\alpha = 15$ (value of the coefficient of increase of the initial dose between the treatment for susceptible strain (D_s) and that for resistant strain (αD_R)) and $\tau = 2$ h (from the time τ, the mortality induced by the D_S treatment is significantly higher than that induced by the αD_R treatment). We set up $a = 20$. The values of the coefficient r (rk^{+1} defined as in Fig. 2), are (——) 0, (— - -) 0.5, (— - -) 1 and (- - - -) 1.5. The tested hypothesis is rejected in the zone for which $t_1 > \tau$.

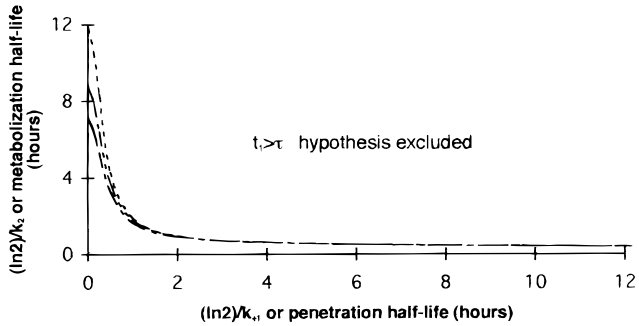

Fig. 7. Limits for which $t_1 = \tau$ as a function of k_{+1} and k_2 with different a values with $a = \alpha$. We assume values of α (value of the coefficient of increase of the initial dose between the treatment for susceptible strain (D_S) and that for resistant strain (αD_R)) is always equal to a and $\tau = 2$ h (from the time τ, the mortality induced by the D_S treatment is significantly higher than that induced by the αD_R treatment). We set up $r = 0$ (rk_{+1} defined as in Fig. 2). The values of the coefficient of increased metabolism a, are (— - -) 15, (— -) 20, and (——) 30. The tested hypothesis is rejected in the zone for which $t_1 > \tau$.

3.5 Influence of τ on $t_1 = \tau$

The parameter τ is obtained from mortality kinetics. The time τ is the time after which the mortality of treatment D_S is significantly higher than that of treatment αD_R. In other words, τ is the time after which the mortalities of susceptible and resistant insects begin to diverge. We consider different values of τ (2 h, 3 h, 4 h and 5 h) with $r = 0$, $\alpha = 15$ and $a = 15$ (Fig. 8). It should be noted that the more τ increases, the larger the zone for which the metabolic hypothesis is conceivable.

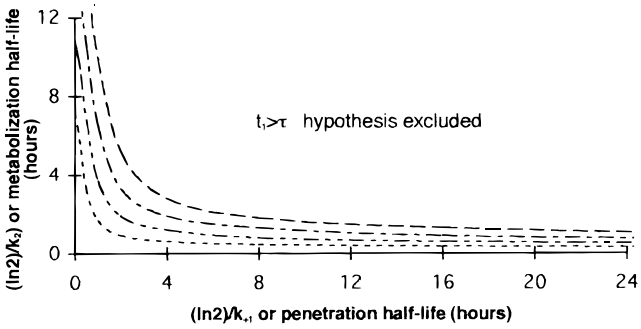

Fig. 8. Limits for which $t_1 = \tau$ as a function of k_{+1} and k_2 with different τ values. We assume values of $\alpha = 15$ (value of the coefficient of increase of the initial dose between the treatment for susceptible strain (D_S) and that for resistant strain (αD_R)). We set up the coefficient of increase of metabolism $a = \alpha = 15$. The value of $r = 0$ (rk_{+1} defined as in Fig. 2). From the time τ, the mortality induced by the D_S treatment is significantly higher than that induced by the αD_R, the values of the coefficient τ are (- - - -) 2 h, (— - -) 3 h, (— -) 4 h and (– –) 5 h. The tested hypothesis is rejected in the zone for which $t_1 > \tau$.

In all cases, the more $(\ln 2)/k_{+1}$ increases (i.e. the more the penetration half-life increases), the smaller the differences between the $t_1 = \tau$ curves, which are obtained for different values of τ, and the lower the value for which $t_1 = \tau$ beyond which the metabolism hypothesis is excluded.

DISCUSSION

The model allows us to study the conditions for which the hypothesis of a resistance mechanism due only to an increased metabolism (i.e. the metabolic hypothesis) is conceivable. The principle of the study is based on the confrontation between the simulation of the insecticide's pharmacokinetics and the mortality kinetics. To judge from our results, the simple comparison between a and α is not sufficient to conclude that the metabolic hypothesis is conceivable. Indeed, similar values of a and α would prompt us to conclude that the metabolic hypothesis is conceivable while the pharmacokinetics of the insecticide could be not consistent with the observed mortality kinetics. It is necessary to take into account the pharmacokinetics of the insecticide and the mortality kinetics (i.e. study t_1 and τ) in order to determine a possible inconsistency between those kinetics, which would allow us to reject the metabolic hypothesis.

The study gives predictable results (e.g. the more a increases, the larger the zone for which the metabolic hypothesis is conceivable). In addition, the fact that a is higher or lower than α modifies considerably the conditions for which the metabolic hypothesis is conceivable. However, the condition $a > \alpha$ is not sufficient to conclude that the metabolic hypothesis is conceivable. In fact, even with $a > \alpha$, there is a zone for which $t_1 = \tau$ and where the metabolic hypothesis is excluded.

The more r increases, the larger the zone for which the metabolic hypothesis is excluded. Thus, the case for which $r = 0$ is the most favourable case for the metabolism hypothesis. Concerning the pyrethroids, the value of r generally varies between 0.5 and 1.5.[14] So, the reversibility of the penetration (i.e. $r > 0$) must be taken into account. It is not favourable for the metabolic hypothesis.

In the studied example in which $\tau = 2$ h, the value of α (or a) with $a = \alpha$ does not greatly modify the $t_1 = \tau$ curves. This means that when $a = \alpha$, the value of α or a does not greatly modify the conditions for which the metabolic hypothesis is conceivable; this is all the more so as $(\ln 2)/k_{+1}$ increases (i.e. the penetration half-life increases). The value of τ considerably modifies the $t_1 = \tau$ curves. The more τ increases, the larger the zone for which the metabolic hypothesis is conceivable. A high value of τ means that it is a long time before the mortality of the susceptible strain is significantly higher than that of the resistant strain. On the other hand, if the mortality of the susceptible strain quickly becomes higher than that of the resistant strain (i.e. low τ) then the conditions are not favourable for the metabolic hypothesis. Thus, a resistance mechanism due only to increased metabolism is consistent with a late decrease of mortality of the resistant strain (i.e. high τ) but not with an early decrease of mortality (i.e. low τ).

For fixed values of τ, α, a and r, the more $(\ln 2)/k_{+1}$ increases (i.e. the slower the penetration of the insecticide), the less favourable the conditions for the metabolic hypothesis. In fact, the more $(\ln 2)/k_{+1}$ increases, the more the $t_1 = \tau$ curve decreases (beyond which the metabolic hypothesis is excluded). The more rapid the metabolism in the susceptible strain, the more the conditions are favourable for the metabolic hypothesis. Indeed, the increase of metabolism in the resistant strain can explain the extent that the mortality, from the time τ, of the resistant strain is significantly lower than that of the susceptible strain only if the metabolism of the susceptible strain is not too slow.

It should be noted that the exclusion of the metabolic hypothesis does not mean that an increase of metabolism does not occur in resistant insects; only that it is unlikely that it is the only mechanism participating in the resistance phenomenon. Similarly, when the metabolic hypothesis is conceivable, it does not necessarily mean that resistance is due only to an increase of metabolism. In addition, by choosing $t_1 < \tau$ to determine the conditions for which the metabolic hypothesis is conceivable, this hypothesis is considerably favoured. Actually, in the zone for which the metabolic hypothesis is conceivable and close to the $t_1 = \tau$ curve, during the time $\tau - \varepsilon$ (with low value of ε) the internal insecticide in the resistant strain is always higher than that in the susceptible strain. During the time ε, the internal insecticide in the susceptible strain is always higher than that in the resistant strain. We are in the zone for which the metabolic hypothesis is conceivable, although unlikely. We prefer never to exclude a correct hypothesis even if some wrong hypothesis remains as conceivable.

This theoretical work may be used to test experimentally the metabolic hypothesis; as in the case of an insecticide for which the pharmacokinetics in the susceptible strain and the increase of metabolism of the insecticide in the resistant strain are known. This gives us the value of k_{+1}, k_2, r and a. First, precise mortality kinetics of the susceptible and resistant strains must be studied experimentally to determine the values of α and τ. Second, in this case, it is possible to test if the metabolic hypothesis is conceivable by comparing t_1 with τ. Also, the test can be done for different values of each of the parameters, taking into account the uncertainty margins.

The model may be modified for different situations. In fact, the model is based on the action mass law, but

there is a case where this law does not apply. Chang and Jordan[17] showed that the higher the initial dose, the lower the penetration of permethrin; the velocity constants thus depend on the dose. In this case, the model must be adaptable to take into account the variation of k_{+1} and r depending on insecticide concentration. However, the principle on which to test the metabolism hypothesis is not modified.

In previous work,[5–7] we used the same principle to test different hypotheses of synergistic mechanisms from experimental data. The model can be adapted to other resistance mechanisms, such as a decrease in penetration associated or not with an increase in metabolism. In those cases, the relation between the internal insecticide, the time and the mortality must be precisely determined.

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